Development of New Carboxylic Acid-Based MMP Inhibitors Derived from Functionalized Propargylglycines

Michael G. Natchus,* Roger G. Bookland, Matthew J. Laufersweiler, Staszek Pikul, Neil G. Almstead, Biswanath De, Michael J. Janusz, Lily C. Hsieh, Fei Gu, Matthew E. Pokross, Vikram S. Patel, Susan M. Garver, Sean X. Peng, Todd M. Branch, Selane L. King, Timothy R. Baker, David J. Foltz, and Glen E. Mieling

Procter and Gamble Pharmaceuticals, 8700 Mason−Montgomery Road, Mason, Ohio 45040

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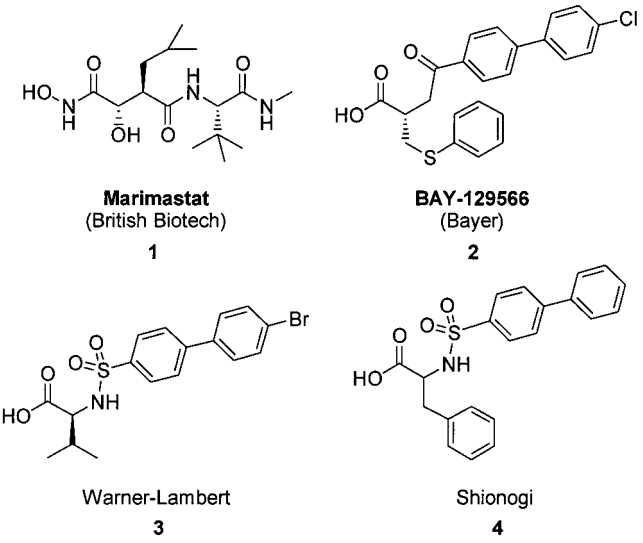
A series of carboxylic acids were prepared from a propargylglycine scaffold and tested for efficacy as matrix metalloproteinase (MMP) inhibitors. Detailed SAR for the series is reported for four enzymes within the MMP family. The inhibitors were typically potent against collagenase-3 (MMP-13) and gelatinase A (MMP-2), while they spared collagenase-1 (MMP-1) and only moderately inhibited stromelysin (MMP-3). Compound **40** represents a typical inhibition profile of a compound with reasonable potency. Introduction of polar groups was required in order to generate inhibitors with acceptable water solubility, and this often resulted in a loss of potency as in compound **63**. High serum protein binding proved to be a difficult hurdle with many compounds such as **48** showing >99% binding. Some compounds such as **64** displayed ∼90% binding, but no reliable method was discovered for designing molecules with low protein binding. Finally, selected data regarding the pharmacokinetic behavior of these compounds is presented.

Introduction

The matrix metalloproteinases (MMPs) are enzymes which are responsible for connective tissue remodeling and have emerged as intriguing targets for a startlingly wide array of disease processes where aberrant tissue remodeling plays a key role.[1] These indications include: osteoarthritis,[2,3] rheumatoid arthritis,[4−6] tumor metastasis,[7−9] multiple sclerosis,[10−12] congestive heart failure,[13−15] and a host of others.

A number of matrix metalloproteinase inhibitors (MMPIs) have progressed into clinical trials for cancer, rheumatiod arthritis, and osteoarthritis. The vast majority of them have been hydroxamic acids such as marimastat (**1**) which is a broad-spectrum inhibitor and was the first MMPI to enter clinical trials for cancer treatment. So far, progression of broad-spectrum MMPIs through these trials has been hampered due to a prevalent side effect known as musculoskeletal syndrome (MSS) which manifests itself as musculoskeletal pain and inflamation. Accordingly, current focus in the field is directed toward the development of selective inhibitors which target only those enzymes which play a role in a given disease state. BAY-129566 (**2**) is a highly selective inhibitor for gelatinase A which showed no signs of MSS in phase 2 clinical trials; however, it was withdrawn from clinical trials. Other groups have also described development of selective, carboxylic acid-based inhibitors including those at Warner-Lambert[16] (**3**) and Shionogi[17,18] (**4**), but so far, no clinical data has been reported.

Our group has been actively developing hydroxamic acid-based inhibitors since the mid-1990s[19−23] and have recently turned our attention to exploring the development of carboxylic acid-based inhibitors. We maintained a desire for selective inhibitors that would target inhibition of MMP-13 while sparing MMP-1 and MMP-7. We chose to begin our investigations with sulfonamide derivatives of unnatural amino acids, and the effort ultimately led us to a functionalized propargylglycine scaffold. We believed that this would allow a diverse set of functionality to be placed on the terminus of the alkyne to access the S2 binding pocket of the MMP enzymes and/or allow for modification of the physical properties of the resulting inhibitors.



Chemistry

Propargylglycine **5** (Scheme 1) served as a convenient starting material for many of the compounds described here as it is commercially available as a racemate as well as in either enantiomeric form. Williams esterification and Boc protection then provided a molecule

* To whom correspondence should be addressed. Tel: (513) 622-3646. Fax: (513) 622-3681. E-mail: natchus.mg@pg.com.

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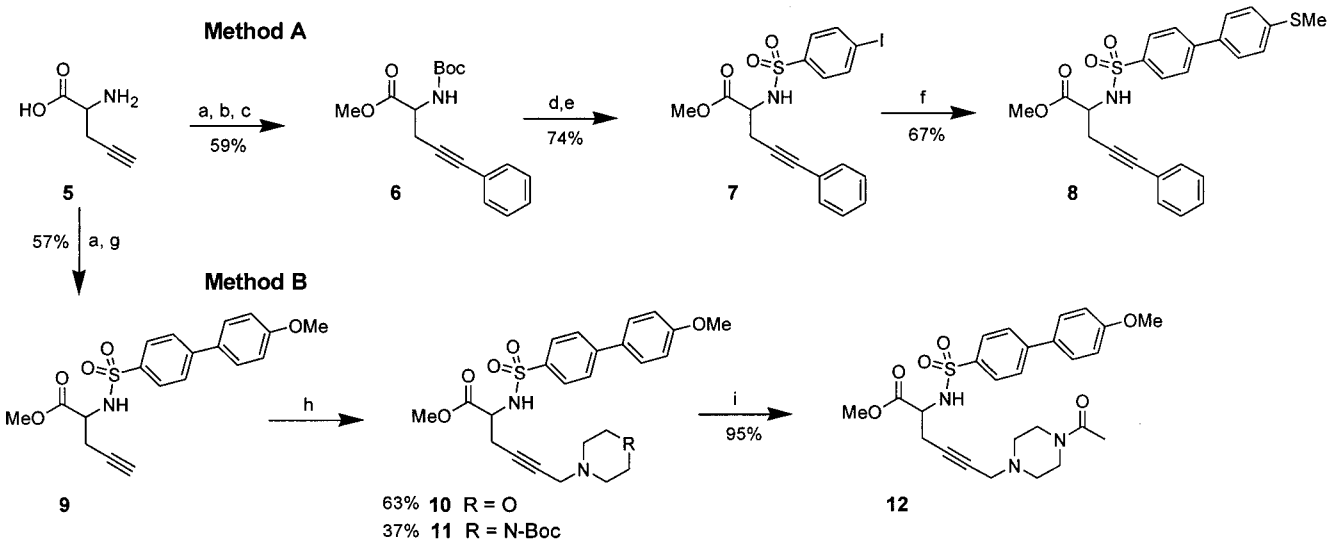
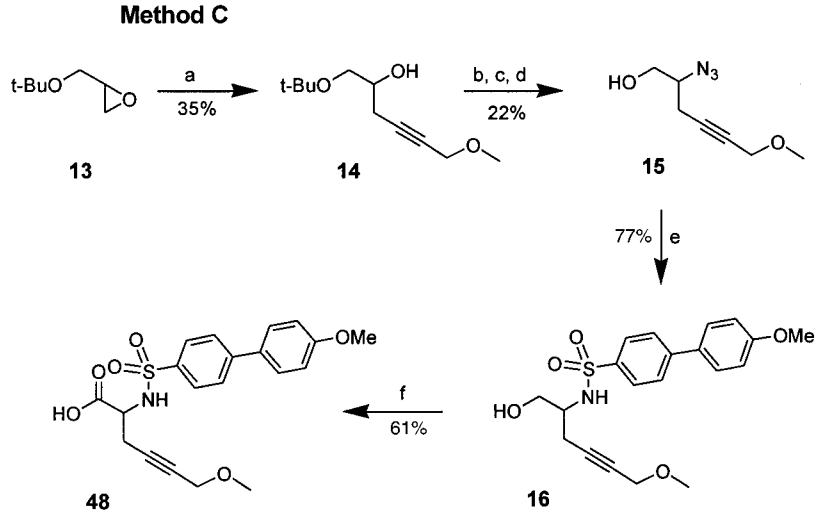
Scheme 1. Palladium-Mediated Couplings of Alkynylglycines[a]



[a] Reagents: (a) MeOH, $SOCl_2$; (b) Boc_2O, CH_2Cl_2, EtN_3; (c) PhI, $(Ph_3P)_4Pd$, CuI, PhH; (d) F_3CCO_2H, CH_2Cl_2; (e) $ClSO_2C_6H_4I$, $NaHCO_3$, dioxane, H_2O; (f) $MeSC_6H_4B(OH)_2$, $(Ph_3P)_4Pd$, $NaHCO_3$, PhH, H_2O; (g) NEt_3, $ClSO_2C_6H_4C_6H_4OMe$, dioxane, H_2O; (h) $(CHO)_n$, Cu(acac)2, toluene, HN⌐R⌙; (i) Ac_2O, CH_2Cl_2, NEt_3.

Scheme 2. Epoxide Opening Route to Alkynylglycines[a]



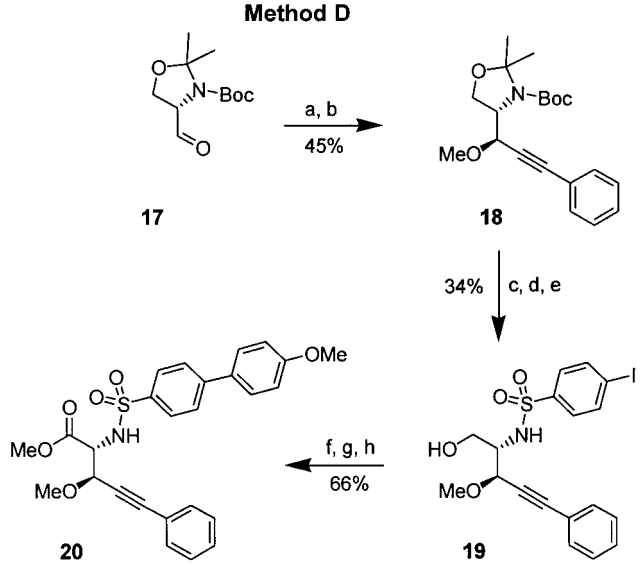
[a] Reagents: (a) $MeOCH_2{\equiv}H$, *n*-BuLi, $BF_3{\cdot}OEt_2$, THF; (b) MsCl, Et_3N, CH_2Cl_2; (c) CF_3CO_2H, CH_2Cl_2; (d) NaN_3, NEt_3, DMF; (e) PPh_3, THF, H_2O, then $ClSO_2C_6H_4{-}C_6H_4OMe$, $NaHCO_3$, H_2O, dioxane; (f) Jones reagent, acetone.

which undergoes an efficient palladium- and copper-mediated coupling with iodobenzene to give **6** in good yield.[24] This coupling method extended well to substituted iodobenzenes but failed with iodo heterocycles despite literature which indicates that various heterocycles couple nicely to alkynes under these conditions.[25] Variously substituted biphenylsulfonamides could then be accessed by removing the Boc protecting group and coupling the resulting free amine with a 4-halobenzenesulfonyl chloride under standard conditions. 4-Iodobenzenesulfonyl chloride was used in this case to give **7**. Suzuki coupling[26] with the resulting sulfonamide **7** and a boronic acid of choice then provided biphenylsulfonamides of type **8** which can be saponified to provide the desired inhibitor **30**.

One can alternatively prepare the desired, optionally substituted methoxybiphenylsulfonyl chloride[27] and then couple it directly to the methyl ester of propargylglycine to give terminal alkyne **9**. A copper-mediated Mannich reaction[25,28] then allowed coupling to various secondary amines such as morpholine and protected piperadines to give propargylamines of type **10** and **11**. The piperadine moiety in **11** could then be deprotected and functionalized to expand the variety of available analogues.

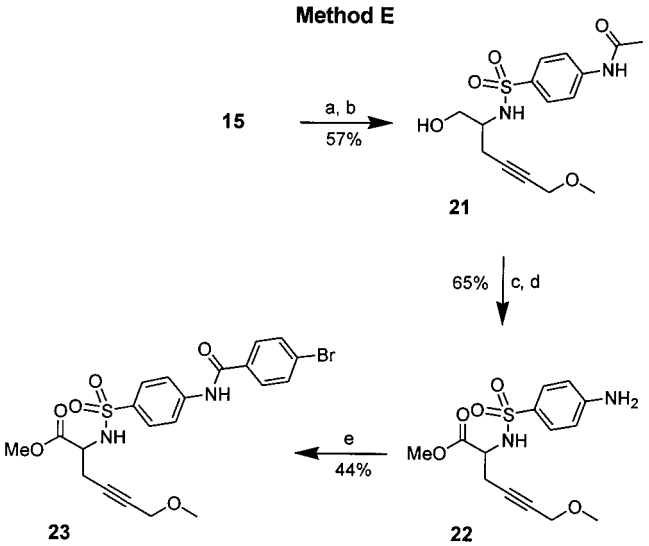
The commercially available epoxide **13** in Scheme 2 provided a second convenient entry to substituted propargylglycines. It can be obtained in either enantiomeric configuration; however, for the purposes of this study, racemic material was used. A Lewis acid-mediated ring opening with an alkyne anion of choice provided chiral alcohols of type **14**. The relative mesylate of **14** was prepared but proved to be inert to azide displacement due to the deactivating effect of the *β*-*tert*-butoxy group. The *tert*-butyl group was therefore removed under acidic conditions, and the resulting *β*-hydroxy mesylate underwent slow but quantitative displacement upon treatment with sodium azide to give azide

Scheme 3. Preparation of β-Alkoxypropargylglycines[a]



[a] Reagents: (a) Ph≡H, *n*-BuLi, THF; (b) NaHMDS, MeI, THF; (c) Amberlyst 15, MeOH; (d) 4 N HCl, dioxane; (e) 4-$IC_6H_4SO_2Cl$, H_2O, Et_3N, dioxane; (f) Jones reagent, acetone; (g) CH_2N_2; (h) 4-$MeS-C_6H_4B(OH)_2$, $Pd(Ph_3)_4$, $NaHCO_3$, PhH.

Scheme 4. Sulfanilylpropargylglycine Formation and Acylation[a]



[a] Reagents: (a) $P(Ph)_3$, THF, H_2O; (b) $ClSO_2C_6H_4NHCOMe$, $NaHCO_3$, H_2O, dioxane; (c) Jones reagent, acetone; (d) MeOH, H_2SO_4; (e) $ClCOC_6H_4Br$, CH_2Cl_2, NEt_3.

15. The most convenient method of proceeding proved to be decomposition of the azide with triphenylphosphine. The resulting amine is then extracted from organic media with dilute acid, neutralized with sodium bicarbonate, and treated with methoxybiphenylsulfonyl chloride under Shotten−Baumann conditions to give the desired sulfonamide **16**. The primary alcohol was then oxidized to provide the desired inhibitor **48**.

The commercially available aldehyde **17** provided an asymmetric and diastereoselective entry into propargylglycines that contained a β-alkoxy group as seen in Scheme 3. This was set up initially with an alkynyllithium addition to **17** which occurred diastereospecifically. Subsequent methylation under basic conditions then gave **18** in high combined yield for the two steps. The remaining transformations were then carried out in analogy with Scheme 1. The free amino alcohol moiety was revealed upon exposure of **18** to acidic Amberlyst 15 resin followed by 4 N HCl to remove the Boc protecting group. The free amine was then trapped with 4-iodobenzenesulfonyl chloride under Schotten−Baumann conditions to deliver the sulfonamide **19**. Finally, the primary alcohol in **19** was oxidized to an acid with Jones reagent and trapped as its methyl ester. Suzuki coupling with 4-methoxybenzeneboronic acid then gave desired oxygen containing scaffold **20**.

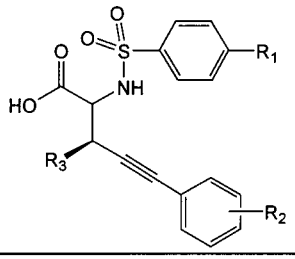
The sulfonamidoaniline moieties can be accessed and functionalized via acetamides of type **21**. This in turn can be accessed from any scaffold of interest which bears a free nitrogen by coupling with a sulfonyl chloride of choice; alternatively, azides of type **15** can be decomposed and trapped as described for compound **16**. This provides an alternative to methods which have been previously described for related compounds.[29,30] Jones oxidation of the free alcohol then provides its carboxylic acid analogue, and subsequent treatment with concentrated sulfuric acid in methanol esterifies the acid and deprotects the anilinic nitrogen to provide **22** as an HCl salt. This then serves as a pivotal intermediate which can be functionalized with any number of acyl equivalents including *p*-anisoyl chloride which was used to provide **23**. Saponification of the ester then gave inhibitor **50**.

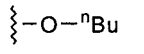
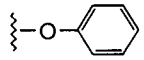
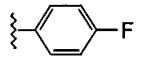
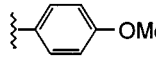
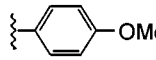
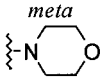
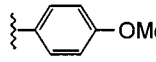
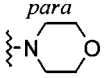
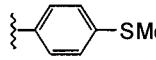
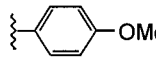
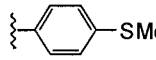
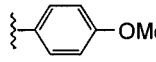
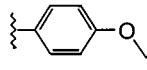
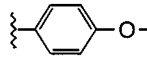
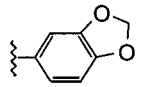
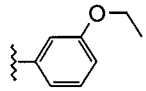
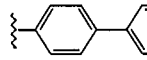
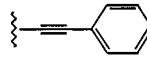
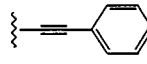
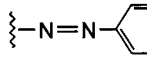
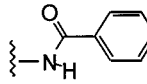
SAR and Biological Results

All compounds were tested for the inhibition of various MMP enzymes, including collagenases-1, -2, and -3 (MMP-1, -8, and -13, respectively), gelatinases A and B (MMP-2 and -9, respectively), stromelysin (MMP-3), and matrilysin (MMP-7). The data for MMP-7, -8, and −9 are omitted here for clarity, but their inhibition profiles tended to mimic MMP-1 and -2, respectively, while MMP-9 was typically 1 order of magnitude less potent than MMP-2. All binding data for the carboxylic acid-based inhibitors in this manuscript were obtained and reported at pH = 7.4. While it has been reported that a dramatic potency increase is observed when data is obtained at a slightly more acidic pH (6.0−6.5),[16] we felt that data obtained at physiological pH was more relevant to the natural disease situation in vivo. The SARs of the substituted propargylglycines seemed to be highly variable and depend very heavily on the substitution pattern of the aromatic sulfonamide group as well as the appendage which was attached to the terminus of the alkyne substituent. Water solubility and serum protein binding data for selected compounds were also obtained and are reported in Table 4.

SAR of Phenylpropargylglycine Derivatives. Much of the early SAR work was done with inhibitors that contain a phenylpropargylglycine scaffold, and these are listed in Tables 1 and 2. These inhibitors typically demonstrated reasonable potency for MMP-2 and -13 in those cases where the P1′ substituent was a substituted biphenylsulfonamide moiety such as with **27**. Potency was rarely observed for MMP-1, while MMP-3 tended to receive moderate inhibiton. A notable exception to this was observed with the insertion of an alkyne into the biphenyl system as in **40** which retained a similar inhibition profile to compound **27**. Replacement of the alkyne with the azido group in **41** resulted

Table 1. Conjugated Phenylalkynes

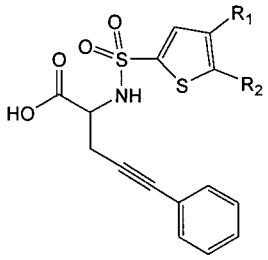


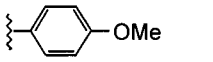
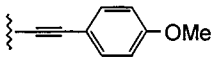
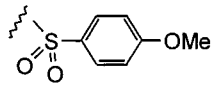
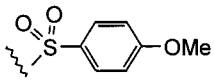
				MMP - IC_{50} (nM)[a]			
#	R_1	R_2	R_3	-1	-2	-3	-13
24		-H	-H	nd	nd	15,400	2,037
25		-H	-H	>10,000	1,480	4,560	2,150
26		-H	-H	5,600	175	8,900	231
27		-H	-H	2,000	12	2,280	21
28		*meta* 	-H	3,660	16	3,520	24
29		*para* 	-H	>10,000	158	14,600	303
30		-H	-H	631	6	3,400	16
31[b]		-H	-OMe	4,200	31	3,700	81
32[b]		-H	-OMe	3,400	8	5,100	33
33[b]		-H	-OBn	>10,000	8	4,270	14
34		-H	-H	>10,000	26	2,428	50
35		-H	-H	>10,000	1,475	4,560	2,146
36		-H	-H	10,300	29	5,440	104
37		-H	-H	>10,000	2391	nd	>1,600
38		-H	-H	>10,000	29	>9,999	59
39		-H	-H	>10,000	19	22,100	89
40		-H	-H	>10,000	9	4,360	35
41		-H	-H	>10,000	87	16,300	498
42		-H	-H	>10,000	6	3,570	127

[a] See Experimental Section for assay protocols. Standard deviations for enzyme assays were typically ±30% of the mean or less. [b] Prepared as a single enantiomer with an *S*-configuration at the α-center. nd, not determined.

in the loss of 1 order of magnitude in potency. In most cases, a thiomethyl group in the 4′-position of the biphenyl moiety seemed to be the optimum substituent for potency although a methoxy group was also attractive (compare compounds **27** vs **30**). Arylamides of type **42** were typically exclusively selective for MMP-2.

Table 2. Substituted Sulfonylthiophenes as P1′ Substituents



#	R_1	R_2	MMP - IC_{50} (nM)[a] -1	-2	-3	-13
43	-H		3,250	20	1,320	33
44	-H		>10,000	46	10,100	145
45	-H		>10,000	>10,000	>10,000	>10,000
46		-H	>10,000	>10,000	>10,000	>10,000

[a] See Experimental Section for assay protocols. Standard deviations for enzyme assays were typically ±30% of the mean or less.

High serum protein binding (see Table 4) proved to be a significant hurdle which needed to be addressed. Data with highly lipophilic compounds such as **27** and **40** indicated that these compounds were highly protein-bound in the blood and thus unavailable as free inhibitors. We hoped to establish a link between lipophilicity and water solubility, so we sought to incorporate more hydrophilic groups into the phenylpropargylglycine scaffold as seen with compounds **32**, **28**, and **34**. Indeed, increased water solubility was observed, but this was not accompanied by a concomitant lowering of protein binding. We thus chose to remove the phenyl ring from the primary scaffold and replaced it with groups containing a greater array of heteroatom diversity.

SAR of Substituted Butynylglycine Derivatives. Replacement of the phenyl ring with a substituted methylene allowed for a far greater diversity of functionality, as seen in Table 3. Initial probing with compound **47** indicated that this methylene homologue of **27** resulted in negligible change in potency. Unfortunately, replacement of the phenyl ring with hydrophilic groups such as methoxy (**48**), phenoxy (**55**), morpholino (**61**), and substituted piperidines (**65**−**67**) all resulted in significant losses in potency. We hoped to overcome this through modification of the P1′ group, and indeed, a 4-fold increase in potency was observed upon replacement of the methoxy group with the thiomethoxy group in **62** and by extending the biphenyl with the acetylene in **63**. As before, replacement of the biphenyl moiety with aromatic amides as in **50** and **64** resulted in highly MMP-2-selective compounds.

As we had hoped, most of the compounds in Table 3 were more water-soluble than the phenyl-containing analogues as exemplified by compounds **48**, **50**, **63**, and **64**, all of which showed water solubility in excess of 5 mg/mL. Except for **48**, a notable decrease in protein binding for all of these compounds was also observed. Inconsistent trends such as **48** vs **49** vs **50** however do not allow us to ascribe a direct correlation between water solubility and protein binding. We ultimately observed that most compounds below 1 mg/mL are highly protein-bound and should be avoided, but no reliable, rational method was discovered for the design of compounds with low protein binding.

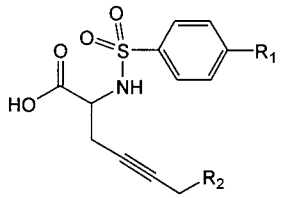
Structure of Inhibitor−Stromelysin Complex of Compound 48.[31] A crystal structure of stromelysin was obtained with compound **48** bound in the active site according to a previously described procedure,[32] and the data is represented graphically in Figures 1 and 2. The structure was obtained by soaking the inhibitor into a previously prepared crystal at pH = 6.5. The data clearly shows that the biphenyl moiety fits into the P1′ pocket of the enzyme. This is consistent with structural data which has been reported previously with carboxylic acid scaffolds containing similar sulfonamide moieties using both X-ray crystal data[33] and homology modeling.[16] Additionally, key hydrogen bonds exist between the internal sulfonamide oxygen and both Ala 165 (3.4 Å) and Leu 164 (2.7 Å) which seems to be a key interaction with sulfonamide types of MMP inhibitors.[22]

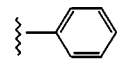
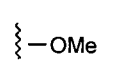
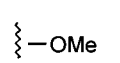
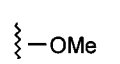
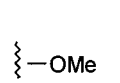
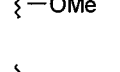
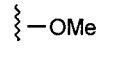
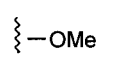
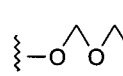
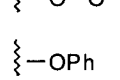
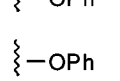
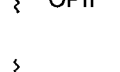
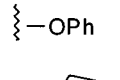
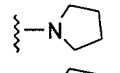
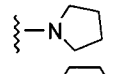
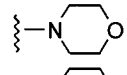
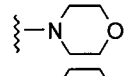
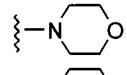
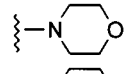
The carboxylic acid binds to the active site zinc ion in what appears to be a bidentate fashion with bonding distances of 1.9 and 2.9 Å. The close proximity of Glu 202 to the ligand carboxylic acid imparts significant anion/anion repulsion when they are both in their ionic forms. This becomes a hydrogen-bonding interaction as the acids are protonated at lower pH as displayed in this structure where the two acids are in hydrogen-bonding distances of 2.5 and 3.2 Å from one another. This explains the dramatic pH dependence displayed by MMP-3 on the potency of carboxylic acid inhibitors.

The alkynyl side chain seems to rest comfortably in the lipophyllic S2 cleft, and there is significant density data to support its preference for this orientation.

In Vivo Pharmacokinetics. The pharmacokinetics of selected representatives from this series of compounds

Table 3. Methylene-Substituted Alkynes



#	R_1	R_2	MMP - IC_{50} (nM)[a] -1	-2	-3	-13
47			3,340	33	777	22
48			6,150	20	4,090	76
49			24,800	17	3,330	147
50			>10,000	8	9,000	486
51			>10,000	6	8,620	270
52			>10,000	567	>10,000	16,900
53			>10,000	2,770	>10,000	>10,000
54			6,510	33	2,340	88
55			>10,000	23	1,810	254
56			>10,000	9	3,920	226
57			>10,000	9	2,850	154
58			>10,000	380	>10,000	3050
59			>10,000	100	>10,000	1660
60			>10,000	297	>10,000	869
61			13,700	104	10,700	478
62			11,500	24	5,060	103
63			>10,000	28	6,600	116
64			>10,000	12	17,400	344
65			>10,000	547	>10,000	774
66			>10,000	661	>10,000	1,691
67			>10,000	1,160	>10,000	2,780

[a] See Experimental Section for assay protocols. Standard deviations for enzyme assays were typically ±30% of the mean or less. nd, not determined.

Table 4. Solubility and Protein Binding of Selected Inhibitors

compd	sol (mg/mL)	protein binding (%)[a]
27	0.02	
28	0.58	
32	0.12	
34		99.7
36	0.08	
40	0.02	
43	0.03	
48	5.8	99.4
49	12	98.7
50	3.2	93.9
56	0.41	
57	0.05	99.7
63	151	96.2
64	4.4	90.7

[a] Average error for this measurement is ±0.2%.

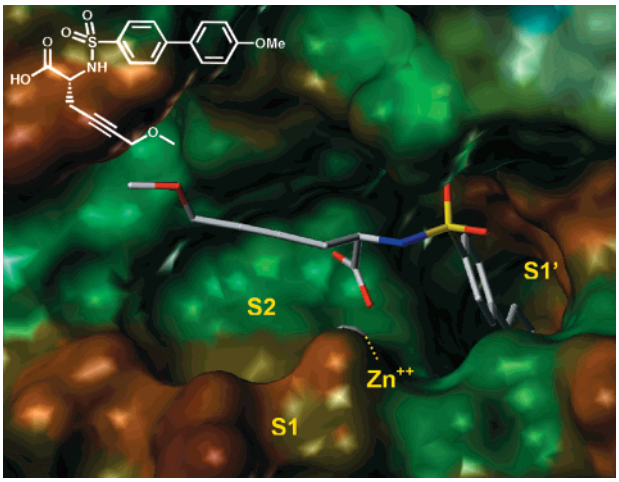


Figure 1. Surface structure of the stromelysin−**48** complex.

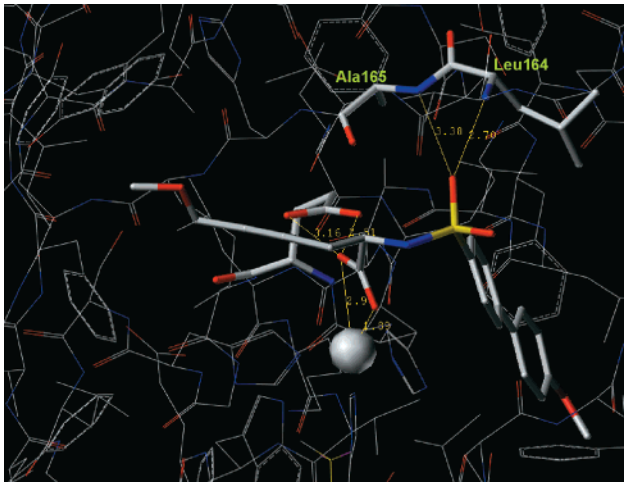


Figure 2. Binding interactions in the stromelysin−**48** complex.

was investigated using the cassette dosing technique,[34−36] and the data for some of these is shown in Table 5. The studies were conducted in fasted rats (n = 3/4) using 4−5 compounds per experiment at a dose of 1−5 mg/kg of each inhibitor. Each animal study included a control compound that had been previously characterized in a single-compound experiment which served to validate each data set and illuminate problems such as saturation of elimination. The data suggest that most of the compounds have a somewhat short half-life with low to moderate bioavailability (BA). We assume that poor absorption may contribute to the lower BA, and this is supported by the correlation we see between BA and molecular weight. The two compounds that have molecular weights over 500, **28** and **63**, have significantly lower BA values than their smaller counterparts, **27** and **40**. We are continuing to investigate the factors which are contributing to both the low BA and short half-life of these compounds.

Table 5. In Vivo Pharmacokinetics of Selected Compounds

compd	BA (%)	$t_{1/2}$ (h)
27	49	1.7
28	7	0.1
40	26	0.7
63	12	3.6

Conclusion

We have described a new series of carboxylic acid-based MMP inhibitors which are derived from a propargylglycine scaffold. The more potent of these compounds are highly selective for MMP-2 and -13 and demonstrate low-nanomolar activity. The more lipophilic compounds tend to be highly bound by serum albumen. The more hydrophilic compounds tend to be less potent; however, some examples exhibit somewhat less protein binding. Limited pharmacokinetic data shows a high variability between compounds.

Experimental Section

General. All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. The following solvents and reagents have been abbreviated: tetrahydrofuran (THF), ethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), methanol (MeOH). All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions were monitored by thin-layer chromatography on 0.25-mm silica gel plates (60F-254, E. Merck) and visualized with UV light, iodine vapors, or 5% phosphomolybdic acid in 95% ethanol. Final compounds were typically purified either by flash chromatography on silica gel (E. Merck, 40−63 mm) or by preparative reverse-phase high-pressure liquid chromatography (RP-HPLC) using a Waters model 4000 Delta Prep instrument equipped with a Waters Symmetry preparative steel column (C-18, 19 m × 300 mm) as the stationary phase. The mobile phase employed 0.1% formic acid with acetonitrile as the organic modifier. Both isocratic and linear gradient methods were used as appropriate and the flow rate was 20 mL/min. Analytical purity was assessed by RP-HPLC using a Waters 600 system equipped with a diode array spectrometer (range 200−400 nm). The stationary phase was a Waters Symmetry C-18 column (4.6 mm × 200 mm). The mobile phase employed 0.1% formic acid with acetonitrile as the organic modifier and a flow rate of 1.0 mL/min. Analytical data is reported as retention time, t_R, in minutes (% acetonitrile, time, flow rate).

^{1}H NMR spectra were recorded on a Varian Unity-300 instrument. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Low-resolution mass spectra (MS) were recorded on a Micromass Platform quadrupole mass spectrometer. Mass spectra were acquired in either the positive or negative ion mode under electrospray ionization (ESI). Combustion analyses were performed internally.

Human synovial proMMP-3 was obtained from Dr. Hideaki Nagase, University of Kansas Medical Center, Kansas City, KS. Human fibroblast proMMP-1, human MMP-9, and human

recombinant MMP-7 catalytic domain were obtained from Dr. Howard Welgus, Jewish Hospital, St. Louis, MO. Human recombinant MMP-8 catalytic domain was obtained from Dr. Harald Tschesche, University Bielefeld, Bielefeld, Germany. Human recombinant proMMP-2 was purified from CHO cells as described below. Human recombinant truncated MMP-3 and truncated MMP-1 were purified from *E. coli* cells as described below. Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 was purchased from Bachem Bioscience, King of Prussia, PA.

General Method A. (±)-Methyl 2-(*tert*-Butoxycarbonylamino)pent-4-ynoate (5a). Racemic propargylglycine (5.00 g, 51.5 mmol) was taken in 100 mL of methanol and treated dropwise with 7 mL of thionyl chloride and the resulting solution was stirred for 18 h. The mixture was evaporated to dryness and the resulting solid was triturated twice with chloroform and suspended in 100 mL of CH_2Cl_2. The mixture was treated with di-*tert*-butyl dicarbonate (11.2 g, 51.5 mmol) in the presence of 15 mL of triethylamine and allowed to stir for 18 h. The resulting mixture was partitioned between EtOAc and 1 N HCl. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to give a syrup which was filtered through a plug of silica with hexanes:Et_2O (3:1) to give 9.6 g (82%) of clear syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H), 2.06 (dd, J = 2.7, 2.7 Hz, 1H), 2.67−2.84 (m, 2H), 3.79 (s, 3H), 4.45−4.55 (m, 1H), 5.37 (br d, J = 7.5 Hz, 1H); ESI$^+$ MS m/z 228.1 $(M + H)^+$.

(±)-Methyl 2-(*tert*-Butoxycarbonylamino)-5-phenylpent-4-ynoate (6). The free alkyne **5a** (8.6 g, 37.9 mmol) was taken in 100 mL of DMF in the presence of 9 mL of triethylamine. Iodobenzene (4.0 mL, 36.0 mmol), tetrakis(triphenylphosphene)palladium (4.37 g, 3.8 mmol) and copper(I) iodide (1.44 g, 7.6 mmol) were then added sequentially at room temperature. and the resulting mixture was allowed to stir for 18 h. The mixture was then partitioned between 1 N HCl and ether. The organic layer was washed 2× with dilute ammonium chloride, 1× with brine and the combined aqueous layers were back extracted with ether. Combined organic layers were dried over $MgSO_4$, filtered and evaporated. The brown residue was adsorbed onto silica and eluted through a flash silica column with hexanes:ether (3:1 to 1:2) to give 8.24 g (72%) of clear syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 9H), 2.93 (ddd, J = 16.8, 4.9 Hz, 1H), 3.01 (dd, J = 17.2, 4.9 Hz, 1H), 3.83 (s, 3H), 4.54−4.64 (m, 1H), 5.42 (br d, J = 7.7 Hz, 1H), 7.28−7.34 (m, 3H), 7.38−7.44 (m, 2H); ESI$^+$ MS m/z 321.1 $(M + NH_4)^+$, 304.1 $(M + H)^+$.

(±)-Methyl 2-(4-Iodobenzenesulfonylamino)-5-phenylpent-4-ynoate (7). The protected amine **6** (3.38 g, 11.2 mmol) was taken in 30 mL of methanol and treated with 3 mL of $SOCl_2$. The resulting mixture was stirred for 1 h at room temperature. and then evaporated to dryness. The resulting solid was taken in 50 mL of dioxane:water (4:1) in the presence of 10 mL of triethylamine and treated with 4-iodobenzenesulfonyl chloride (4.05 g, 13.4 mmol). The resulting mixture was stirred for 18 h and then partitioned between 1 N HCl and EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to give a gummy residue which was adsorbed onto silica and eluted through a flash silica column with hexanes:ether (7:3 to 4:6) to give 3.9 g (74%) of pale yellow gum which solidified upon standing: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.90 (ddd, J = 17.0, 5.3 Hz, 1H), 2.97 (dd, J = 17.0, 4.9 Hz, 1H), 3.69 (s, 3H), 4.23 (ddd, J = 9.0, 4.9, 4.9 Hz, 1H), 5.58 (d, J = 9.0 Hz, 1H), 7.29−7.38 (m, 5H), 7.61 (ddd, J = 8.6, 2.4, 1.8 Hz, 2H), 7.85 (ddd, J = 8.6, 2.2, 1.8 Hz, 2H); ESI$^+$ MS m/z 486.9 $(M + NH_4)^+$, 469.8 $(M + H)^+$.

Methyl 2-{[4′-Thiomethoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-5-phenylpent-4-ynoate (8). The iodophenylsulfonamide **7** (1.0 g, 2.13 mmol) was dissolved in 13 mL of benzene. Na_2CO_3 (452 mg) was dissolved in 2 mL of water and added, followed by Pd $(PPh_3)_4$ under argon. The 4-thiomethoxyboronic acid (540 mg, 3.2 mmol) in was dissolved in 1.1 mL of MeOH, added and the mixture and refluxed overnight. The cooled reaction was added to Et_2O and washed with water. The organic layer was evaporated, then taken up in EtOAc and washed with 1 N HCl followed with brine, dried over Na_2SO_4, filtered and evaporated. The product was recrystallized from hexane/EtOAc to give 665 mg of powder (67%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.53 (s, 3H), 2.77−2.80 (d, J = 7.3 Hz, 1H), 2.79−2.81 (d, J = 6.0 Hz,1H), 3.48 (s, 3 H), 4.07−4.15 (dd, J = 7.1, 15.6 Hz, 2H), 7.31 (s, 4 H), 7.37−7.39 (d, J = 8.3 Hz, 2 H), 7.54−7.66 (m, 3 H), 7.80−7.83 (d, J = 8.8 Hz, 2H), 7.85−7.88 (d, J = 8.1 Hz, 2H), 8.66−8.70 (d, J = 8.6 Hz, 1H); ESI$^+$ MS m/z 452.0 $(M + H)^+$.

2-{[4′-Thiomethoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-5-phenylpent-4-ynoic Acid (30). The methyl ester **8** (665 mg, 1.43 mmol) was taken up in 75 mL of MeOH and treated with KOH (1.43 g) in 8.5 mL of water and stirred at 25 °C overnight. The material was evaporated to remove MeOH, then partitioned between EtOAc and 1 N HCl (2×) and brine (1×), dried over $MgSO_4$, filtered and evaporated. The solid was recrystallized from EtOAc:hexane to give 494 mg of powder (77%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.53 (s, 3H), 2.71−2.83 (m, 2H), 4.01−4.06 (dd, J = 14.3, 7.3 Hz, 1H), 7.31 (s, 4H), 7.36−7.38 (d, J = 8.2 Hz, 2H), 7.59−7.65 (m, 3H), 7.78−7.80 (d, J = 8.1 Hz, 2H), 7.87−7.90 (d, J = 8.4 Hz, 2H), 8.46−8.49 (d, J = 8.6 Hz, 1H); ESI$^+$ MS m/z 452.0 $(M + H)^+$, 469.0 $(M + NH_4)^+$. Anal. ($C_{24}H_{21}NO_4S_2$) C, H, N.

General Method B. Methyl 2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}aminopent-4-ynoate (9). Racemic propargylglycine (3.43 g, 30.3 mmol) was taken in 100 mL of methanol and treated with 5 mL of thionyl chloride. The resulting mixture was allowed to stir for 16 h at room temperature and then evaporated to dryness and triturated with chloroform. The resulting white solid was taken in 100 mL of dioxane water (4:1) in the presence of 15 mL of triethylamine and treated with [4′-methoxy(1,1′-biphenyl)-4-yl]sulfonyl chloride (7.84 g, 28.8 mmol) and the resulting solution was stirred at room temperature for 16 h and then partitioned between EtOAc and 1 N HCl. The organic layer was washed with 1 N HCl, then brine, then dried over $MgSO_4$, filtered and evaporated to give a white solid which was recrystallized from hexanes:EtOAc to give 6.13 g (57%) of the desired material as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 9H), 2.15 (dd, J = 2.6, 2.6 Hz, 1H), 3.08 (ddd, J = 17.6, 9.5, 2.9 Hz, 1H), 3.18 (dd, J = 17.6, 5.9, 2.9 Hz, 1H), 3.82 (s, 3H), 3.88 (s, 3H), 5.40 (dd, J = 9.2, 5.9 Hz, 1H), 7.09 (br d, J = 8.8 Hz, 2H), 7.63 (br d, J = 8.8 Hz, 2H), 7.74 (br d, J = 8.4 Hz, 2H), 8.18 (br d, J = 8.8 Hz, 2H); ESI$^+$ MS m/z 391.1 $(M + NH_4)^+$, 374.1 $(M + H)^+$.

Methyl 2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}-amino-6-(morpholin-1*N*-yl)hex-4-ynoate (10). Paraformaldehyde (465 mg, 15.3 mmol) and morpholine (1.42 mL, 14.23 mmol) were suspended in 30 mL of dioxane and heated until the solution was clear, then allowed to stir at room temperature for 20 min, at which time pentynoate **9** (4.82 g, 12.9 mmol) and $Cu(acac)_2$ (3.71 g, 14.2 mmol) were added and the solution was heated to 90 °C for 18 h. The reaction mixture was allowed to cool to room temperature and the dioxane was evaporated in vacuo. The residue was then dissolved in ethyl acetate, washed with 5% $NaHCO_3$ (2×), 5% NH_4Cl (2×), brine, dried with $MgSO_4$, filtered and evaporated to give 2.42 g of brown oil. The crude oil was purified via column chromatography with Hex:EtOAc (3:1 to 4:6) to give 4.57 g (63%) of a yellow oil which solidified upon standing: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.52−2.55 (m, 4H), 2.75−2.78 (m, 2H), 3.24−3.26 (m, 2H), 3.60 (s, 3H), 3.74−3.77 (m, 4H), 3.88 (s, 3H), 4.12−4.18 (m, 1H), 7.01−7.04 (d, J = 9.0 Hz, 1H), 7.45−7.60 (m, 4H), 7.66−7.75 (m, 3H), 7.89−7.92 (d, J = 8.6 Hz, 1H); ESI$^+$ MS m/z 473.0 $(M + H)^+$.

Methyl 2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}-amino-6-[4*N*-(*tert*-butoxycarbonyl)piperazin-1*N*-yl)hex-4-ynoate (11). Paraformaldehyde (143 mg, 4.7 mmol) and 4*N*-*tert*-butoxycarbonylpiperazine (814 mg, 4.37 mmol) were suspended in dioxane and heated until the solution was clear, then allowed to stir at room temperature for 20 min, at which time **9** (1.48 g, 3.97 mmol) and $Cu(acac)_2$ (1.14 g, 4.37 mmol) were added and the solution was heated to 90 °C for 18 h. The reaction mixture was allowed to cool to room temperature and the dioxane was evaporated in vacuo. The residue was

then dissolved in ethyl acetate, washed with 5% $NaHCO_3$ (2×), 5% NH_4Cl (2×), brine, dried with $MgSO_4$, filtered and evaporated to give a brown oil (2.42 g). The crude oil was purified via column chromatography with Hex:EtOAc (3:1 to 4:6) to give a yellow oil which solidified upon standing (850 mg, 37%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9H), 2.49−2.69 (m, 6H), 3.39 (br s, 6H), 3.58 (d, J = 13.6 Hz, 1H), 3.78 (s, 3H), 3.89 (s, 3H), 4.84 (d, J = 9.9 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 7.3 Hz, 2H), 7.97 (d, J = 8.1 Hz, 2H); ESI$^+$ MS m/z 572.2 (M + H)$^+$.

2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-6-[4*N*-(*tert*-butoxycarbonyl)piperazin-1*N*-yl)-hex-4-ynoic Acid (66). Compound **11** (128 mg, 0.224 mmol) was dissolved in a 1:1:1 H_2O:methanol:THF solution (15 mL) and NaOH:H_2O 1:1 (1.5 mL) was added dropwise at room temperature. The reaction stirred for 1.5 h until complete by TLC. The solvent was removed in vacuo and the white residue was dissolved in H_2O and passed through a reverse phase silica column (H_2O−CH_3CN:H_2O 1:1) to yield a pure white solid (109 mg, 84%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.39 (s, 9H), 2.32 (br s, 4H), 3.17 (br s, 4H), 3.83 (s, 2H), 4.32−4.35 (m, 1H), 5.17 (s, 1H), 7.07 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H); ESI$^+$ MS m/z 558.2 (M + H)$^+$, 580.2 (M + Na)$^+$. Anal. ($C_{29}H_{30}N_3NaO_5S$·2NaOH) C, H, N.

Methyl 2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}-amino-6-(4*N*-acetylpiperazin-1*N*-yl)hex-4-ynoate (12). Compound **11** (210 mg, 0.37 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (4 mL) was added at room temperature. The reaction stirred for 2 h and the solvent and TFA were stripped off and the resultant residue dissolved in CH_2Cl_2 (5 mL) and to this was added triethylamine (3 mL), and acetic anhydride (38 μL, 0.4 mmol) at room temperature and this stirred 16 h and was then partitioned between ethyl acetate and aqueous 5% $NaHCO_3$. The organic layer was washed with brine, dried with $MgSO_4$, filtered and evaporated to a yellow oil which was purified via column chromatography on silica gel (ethyl acetate−5% methanol:ethyl acetate) to yield a pure white solid (180 mg, 95%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.10 (s, 3H), 2.49−2.68 (m, 5H), 3.45−3.71 (m, 6H), 3.79 (s, 3H), 3.89 (s, 3H), 4.83 (dd, J = 9.9, 3.7 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 7.7 Hz, 2H); ESI$^+$ MS m/z 514.1 (M + H)$^+$.

2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-6-(4*N*-acetylpiperazin-1*N*-yl)hex-4-ynoic Acid (68). The ester **12** was saponified to give the title acid as described for compound **66**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.97 (s, 3H), 2.24−2.42 (m, 6H), 3.17−3.31 (m, 4H), 3.84 (s, 3H), 4.31 (d, J = 9.3 Hz, 1H), 5.18 (s, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H); ESI$^+$ MS m/z 522.1 (M + Na)$^+$, 500.1 (M + H)$^+$. Anal. ($C_{28}H_{34}N_3NaO_7S$·0.7NaOH) C, H, N.

General Method C. 1-*tert*-Butoxy-(2*S*)-hydroxy-6-methoxyhex-4-yne (14). The 1-methoxyprop-2-yne (17.5 mL, 207 mmol) was taken in 350 mL of THF under a N_2 atmosphere and cooled to −78 °C. A solution of *n*-butyllithium (91 mL, 227 mmol) was added slowly via syringe and the resulting solution was allowed to stir for 10 min; afterwhich, borontrifluoride etherate (28.3 mL,227 mmol) was added via syringe and the resulting solution was allowed to stir for 10 min. The neat starting epoxide **13** (26.2 mL, 207 mmol) was added to the solution slowly via syringe and the resulting solution was allowed to stir for 1 h, slowly come to room temperature over 2 h, and then partitioned between hexanes:EtOAc (2:1) and water. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to give a dark red residue which was adsorbed onto silica and eluted through a flash silica column with hexanes:EtOAc (10:1 to 2:1) to give 15.3 g (35%) of clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22 (s, 9H), 1.62−1.69 (br s, 1H), 2.47−2.53 (m, 2H), 3.37 (dd, J = 9.0, 6.4 Hz, 1H), 3.51 (dd, J = 9.0, 3.8 Hz, 1H), 3.82−3.91 (m, 1H), 4.11 (dd, J = 2.1, 2.1 Hz, 2H); ESI$^+$ MS m/z 211.1 (M + H)$^+$.

1-*tert*-Butoxy-(2*S*)-methanesulfonoxy-6-methoxyhex-4-yne (14a). The alcohol **14** (10.5 g, 50 mmol) was taken in 150 mL of CH_2Cl_2 in the presence of 15 mL of Et_3N and treated dropwise with methanesulfonyl chloride (7.74 mL, 100 mmol). The resulting solution was stirred at room temperature for 18 h and then partitioned between CH_2Cl_2 and 1 N HCl. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was adsorbed onto silica and eluted through a column of flash silica with hexanes:EtOAc (2:1 to 1:3) to give 4.71 g (34%) of the desired material as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (s, 9H), 2.72 (dd, J = 2.2, 2.2 Hz, 1H), 2.74 (dd, J = 2.2, 2.2 Hz, 1H), 3.12 (s, 3H), 3.39 (s, 3H), 3.60 (dd, J = 10.3, 6.6 Hz, 1H), 3.65 (dd, J = 10.3, 4.4 Hz, 1H), 4.11 (dd, J = 2.1 Hz, 2H), 4.75 (ddd, J = 12.8, 6.4, 4.4 Hz, 1H); ESI$^+$ MS m/z 296.1 (M + NH_4)$^+$.

1-Hydroxy-(2*S*)-methanesulfonoxy-6-methoxyhex-4-yne (14b). The substrate **14a** (4.68 g, 16.8 mmol) was taken in 100 mL of CH_2Cl_2 and treated dropwise with 10 mL of TFA. The resulting mixture was stirred for 18 h and then evaporated to dryness and triturated 2× with $CHCl_3$ to give 3.3 g of brownish syrup which was carried forward without purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.67−2.82 (m, 2H), 3.15 (s, 3H), 3.38 (s, 3H), 3.85 (dd, J = 12.5, 6.2 Hz, 1H), 3.95 (dd, J = 12.5, 3.3 Hz, 1H), 4.09 (dd, J = 4.0, 4.0 Hz, 2H), 4.81 (ddd, J = 12.8, 6.4, 3.5 Hz, 1H); ESI$^+$ MS m/z 240.1 (M + NH_4)$^+$, 223.1 (M + H)$^+$.

(2*R*)-Azido-6-methoxyhex-4-yn-1-ol (15). The starting crude mesylate **14b** (3.3 g) was taken in 50 mL of DMF in the presence of sodium azide (10.9 g, 168 mmol) and stirred at 60 °C for 42 h. The resulting mixture was then partitioned between Et_2O and water. The organic layer was washed 2× with water and 1× with brine. The aqueous layer was back extracted and the combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a brown syrup which was adsorbed onto silica and eluted through a column of flash silica with hexanes:Et_2O (8:1 to 1:2) to give 1.83 g (64% from **14a**) of the desired product as a pale tan syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.55 (dd, J = 3.3, 3.3 Hz, 1H), 2.57 (dd, J = 2.1, 2.1 Hz, 1H), 3.35 (s, 3H), 3.60−3.72 (m, 2H), 3.72−3.82 (m, 1H), 4.71 (dd, J = 2.0, 2.0 Hz, 2H); ESI$^+$ MS m/z 170.1 (M + H)$^+$.

(2*R*)-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-6-methoxyhex-4-yn-1-ol (16). The starting azide (800 mg, 4.73 mmol) was taken in 25 mL of THF and triphenylphosphene (2.50 g, 9.47 mmol) was added followed by dropwise addition of 3 mL of water which caused mild effervescing. The mixture was stirred for 1 h at which time the mixture was diluted with 200 mL of hexanes:EtOAc (1:1) and extracted twice with 1 N HCl. The organic layer was diluted with 50 mL of dioxane and neutralized with excess, solid sodium carbonate. [4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl chloride (1.93 g, 7.10 mmol was added and the mixture allowed to stir for 16 h. The mixture was then partitioned between 1 N HCl and EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was then chromatographed over flash silica with hexanes:EtOAc (1:1 to 1:4) to give 1.42 g (77%) of the desired sulfonamide: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.45 (dddd, J = 16.9, 6.6, 2.0, 2.0 Hz, 1H), 2.53 (dddd, J = 16.9, 5.1, 2.2, 2.2 Hz, 1H), 3.45−3.56 (m, 1H), 3.67 (dd, J = 11.4, 4.8 Hz, 1H), 3.75 (dd, J = 11.0, 5.1 Hz, 1H),3.88 (s, 3H), 4.00 (dd, J = 2.0, 2.0 Hz, 2H), 5.38 (d, J = 7.7 Hz, 1H), 7.02 (ddd, J = 8.8, 2.9, 2.2 Hz, 2H), 7.58 (ddd, 8.8, 2.9, 2.2 Hz, 2H), 7.70 (br d, J = 8.1 Hz, 2H), 7.95 (br d, J = 8.4 Hz, 2H); ESI$^+$ MS m/z 407.2 (M + NH_4)$^+$, 390.1 (M + H)$^+$.

(2*R*)-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-6-methoxyhex-4-ynoic Acid (48). Alcohol **16** (1.15 g, 2.95 mmol) was dissolved in acetone and Jone's reagent (10 mL, 8 N) was added dropwise at room temperature. The reaction stirred for 4 h and was then quenched with isoproanol. The green precipitate was filtered off through Celite and the solvent was then evaporated to a green residue which was dissolved in 5% aqueous $NaHCO_3$ and washed with EtOAc. The aqueous layer was acidified with concentrated HCl and the pure white precipitate filtered off and washed with water and dried to give 720 mg (61%) of a white powder: ^{1}H NMR

(DMSO-d_6, 300 MHz) δ 2.51 (s, 3H), 3.17 (s, 2H), 3.82 (s, 3H), 3.85−3.93 (m, 3H), 7.07 (d, J = 9.0 Hz, 2H), 7.70 (d, J = 8.6 Hz, 2H), 7.82 (s, 4H), 8.34 (d, J = 8.6 Hz, 1H), 12.9 (s, 1H); ESI^- MS m/z 402.0 $(M - H)^-$. Anal. ($C_{20}H_{21}NO_6S$) C, H, N.

General Method D. *tert*-Butyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-hydroxy-3′-phenylprop-2′-ynyl)oxazolidine-3-carboxylate (18a). A solution of phenylacetylene (1.73 g, 17 mmol) in THF (75 mL) was cooled to −78 °C and then *n*-butyllithium (2.5 M, 6.3 mL, 15.7 mmol) was added. The resulting mixture was warmed to −20 °C and stirred for 15 min. The mixture was recooled to −78 °C and then *tert*-butyl (*S*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (**17**; 3 g, 13.1 mmol) in THF (10 mL) was slowly added. The mixture was then warmed to −20 °C and stirred for 30 min. The reaction was quenched by the addition of saturated ammonium chloride solution (30 mL). The mixture was then poured into water and extracted with methylene chloride. The organic extracts were dried ($MgSO_4$) and then concentrated to an oil under reduced pressure. The resulting oil was purified by chromatography on silica gel using 9:1 hexane:EtOAc as the eluent to provide 2.20 g (51%) of the desired compound as a pale oil. A small quantity of the minor, undesired diastereomer (0.1 g, 2.3%) was also obtained in the reaction: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.52 (s, 9 H), 1.55 (s, 3 H), 1.62 (s, 3 H), 4.01 (m, 1 H), 4.20 (m, 1 H), 4.25 (m, 1 H), 4.77 (m, 1 H), 5.32 (m, 1 H), 7.34 (m, 3 H), 7.42 (m, 2 H); ESI^+ MS m/z 332 $(M + H)^+$.

***tert*-Butyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-methoxy-3′-phenylprop-2′-ynyl)oxazolidine-3-carboxylate (18).** The alcohol **18a** (10.04 g, 30.3 mmol) in THF (150 mL) was stirred at 0 °C and then sodium hexamethyldisilazide (0.6 M, 60 mL, 36.4 mmol) was added. The mixture was stirred at 0 °C for 15 min. and then iodomethane (4.73 g, 33.3 mmol) was added. The resulting mixture was stirred at room temperature overnight and then the reaction was quenched by the addition of saturated ammonium chloride solution (20 mL). The mixture was poured into water and then extracted with methylene chloride. The organic extracts were dried (Na_2SO_4) and then concentrated to an oil under reduced pressure. Purification of the oil was accomplished by chromatography on silica gel using 9:1 hexane:EtOAc as the eluent to provide the desired product (9.25 g, 88%) as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.52 (s, 9 H), 1.57 (s, 3 H), 1.66 (s, 3 H), 3.52 (s, 3 H), 4.02 (m, 1 H), 4.23 (m, 1 H), 4.45 (m, 1 H), 4.69 (m, 1 H), 7.32 (m, 3 H), 7.44 (m, 2 H); ESI^+ MS m/z 346 $(M + H)^+$.

***tert*-Butyl (1*S*,2*R*)-*N*-[2-Methoxy-1-(hydroxymethyl)-4-phenylbut-2-ynyl]carbamate (19a).** The acetonide **18** (9.22 g, 26.7 mmol) in methanol (150 mL) was stirred at room temperature and then Amberlyst 15 (15 g) was added. The resulting heterogeneous mixture was stirred at room temperature for 24 h. The mixture was filtered through Celite with the aid of methanol. The product was purified by chromatography on silica gel using 7:3 hexane:EtOAc as the eluent. The product was obtained (5.03 g, 62%) as a colorless oil which solidified to a white solid upon standing: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 9 H), 2.97 (br s, 1 H), 3.49 (s, 3 H), 3.77 (dd, J = 4.8, 11.6, 1 H), 4.01 (m, 1 H), 4.17 (m, 1 H), 4.48 (m, 1 H), 7.34 (m, 3 H), 7.46 (m, 2 H); ESI^+ MS m/z 306 $(M + H)^+$.

(1*S*,2*R*)-2-Methoxy-1-(hydroxymethyl)-1-amino-4-phenylbut-2-yne (19b). The carbamate **19a** (5.0 g, 16.4 mmol) in dioxane was stirred at room temperature and then 4 N HCl (15 mL) was added. The resulting mixture was stirred at room temperature for 14 h. The reaction mixture was made basic by the addition of saturated sodium bicarbonate solution. The resulting mixture was extracted with methylene chloride. The organic extracts were dried ($MgSO_4$) and then concentrated to an oil (3.38 g, quantitative) under reduced pressure. No further purification of the oil was performed: ^1H NMR ($CDCl_3$, 300 MHz) δ 2.53 (br s, 3 H), 3.13 (m, 1 H), 3.49 (s, 3 H), 3.74 (m, 2 H), 4.22 (m, 1 H), 7.33 (m, 3 H), 7.47 (m, 2 H); ESI^+ MS m/z 206 $(M + H)^+$.

(2*R*)-Methoxy-1-(hydroxymethyl)-(1*S*)-(4′-iodophenylsulfonyl)amino-4-phenylbut-2-yne (19). The amino alcohol **19b** (3.30 g, 16.1 mmol) in dioxane (30 mL) and water (30 mL) was stirred at room temperature and then triethylamine (3.25 g, 32.2 mmol) followed by pipsyl chloride (5.3 g, 17.7 mmol) were added. The resulting mixture was stirred at room temperature overnight. The reaction was diluted with 1 N HCl and then extracted with methylene chloride. The organic extracts were dried and then concentrated to an oil (4.20 g, 55%) under reduced pressure: ^1H NMR ($CDCl_3$, 300 MHz) δ 2.58 (br s, 1 H), 3.40 (s, 3 H), 3.57 (m, 2 H), 4.11 (m, 1 H), 4.39 (m, 1 H), 5.77 (d, J = 8.4, 1 H), 7.38 (m, 3 H), 7.45 (m, 2 H), 7.67 (d, J = 8.8, 2 H), 7.87 (d, J = 8.4, 2 H), ESI^+ MS m/z 472 $(M + H)^+$.

Methyl (2*R*)-(4′-Iodophenylsulfonyl)amino-(3*S*)-methoxy-5-phenylpent-4-ynoate (20a). The alcohol **19** (2.0 g, 4.24 mmol) in acetone (100 mL) was stirred at room temperature and then the Jones reagent (8N, 30 mL, excess) was slowly added. The resulting mixture was stirred at room temperature for 4 h and then the reaction was quenched by the addition of 2-propanol. A green precipitate formed after the mixture was stirred for 30 min. The solution was then filtered through Celite with the aid of acetone. The filtrate was concentrated to an oil under reduced pressure. The oil was dissolved in methanol and then an ethereal solution of diazomethane was added. The mixture became slightly yellow when excess diazomethane had been added. The mixture was concentrated to a light yellow solid. Purification of the solid was accomplished by chromatography on silica gel using 8:2 hexane:EtOAc as the eluent to provide the product (1.70 g, 82%) as a yellow solid: ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (s, 3 H), 3.66 (s, 3 H), 4.37 (m, 1 H), 4.51 (d, J = 4.2, 1 H), 7.34 (m, 3 H), 7.40 (m, 2 H), 7.61 (d, J = 8.4, 2 H), 7.88 (d, J = 8.8, 2 H); ESI^+ MS m/z 500 $(M + H)^+$.

Methyl (2*R*)-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}-amino-(3*S*)-methoxy-5-phenylpent-4-ynoate (20). The starting sulfonamide **20a** (510 mg, 1.02 mmol) and 4-methoxyphenylboronic acid (230 mg, 1.53 mmol) were dissolved in 10 mL of benzene, 1.5 mL of EtOH and 1.5 mL of water in the presence of $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) and 200 mg of Na_2CO_3 and brought to reflux for 18 h. The mixture was cooled to room temperature, poured into water, and extracted with methylene chloride. The organic layer was dried ($MgSO_4$), filtered and evaporated. The crude product was purified by silica gel chromatography using 9:1 hexane:EtOAc to give the desired product (0.40 g, 81%) as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 3.43 (s, 3 H), 3.62 (s, 3 H), 3.89 (s, 3 H), 4.39 (dd, J = 4.2, 10.1, 1 H), 4.52 (d, J = 4.2, 1 H), 5.60 (d, J = 9.6, 1 H), 7.03 (m, 2 H), 7.30 (m, 3 H), 7.58 (d, J = 8.8, 2 H), 7.69 (d, J = 8.7, 2 H), 7.93 (d, J = 8.8, 2 H); ESI^+ MS m/z 480 $(M + H)^+$.

(2*R*)-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-(3*S*)-methoxy-5-phenylpent-4-ynoic Acid (31). The methyl ester **20** (350 mg, 0.73 mmol) was dissolved in water:methanol:THF (5 mL:5 mL:5 mL) and then lithium hydroxide (1 g, excess) was added. The resulting mixture was stirred overnight at room temperature. The reaction was acidified with 1 N HCl and then the mixture was extracted with methylene chloride. The organic extracts were dried ($MgSO_4$) and then concentrated to an oil under reduced pressure. Purification was accomplished on reverse phase HPLC using a gradient from water to acetonitrile. The product was obtained as a white powder (0.25 g, 74%): ^1H NMR ($CDCl_3$, 300 MHz) δ 3.42 (s, 3 H), 3.90 (s, 3 H), 4.40 (dd, J = 4.2, 10.1, 1 H), 4.53 (d, J = 4.2, 1 H), 5.62 (d, J = 9.6, 1 H), 7.02 (m, 2 H), 7.33 (m, 3 H), 7.59 (d, J = 8.8, 2 H), 7.70 (d, J = 8.7, 2 H), 7.92 (d, J = 8.8, 2 H); ESI^+ MS m/z 464 $(M - H)^-$. Anal. ($C_{25}H_{23}NO_6S \cdot H_2O$) C, H, N.

General Method E. 2-(4-Acetamidobenzenesulfonyl)-amino-6-methoxyhex-4-yn-1-ol (21). PPh_3 (10.4 g, 39.6 mmol) and H_2O (5 mL), were added to a solution of **15** (2.68 g, 15.8 mmol) in THF (30 mL). The solution stirred at room temperature with vigorous bubbling for 3.5 h, then was dilluted with EtOAc/Hex (2:1) and extracted with 0.5 N HCl (2 × 30 mL). The acidic layer was basified with Na_2CO_3 and dilluted with 60 mL of 1,4-dioxane. Sulfanilyl chloride (3.7 g, 15.8 mmol) was added and the reaction stirred at room temperature for 16 h. The dioxane was evaporated and the

solution extracted with EtOAc. The EtOAc layer was washed with 1 N HCl, dried with $MgSO_4$, filtered and evaporated to a sticky white solid (3.1 g, 57%) which was used without further purification: ^{1}H NMR (CD_3OD, 300 MHz) δ 2.18 (s, 3H), 2.35 (ddd, J = 16.8, 6.0, 2.1 Hz, 1H), 2.48 (ddd, J = 16.9, 6.2, 2.1 Hz, 1H), 3.32−3.38 (m, 4H), 3.49 (dd, J = 11.0, 6.4 Hz, 1H), 3.37 (dd, J = 11.0, 5.0 Hz, 1H), 3.97 (s, 2H), 7.77 (d, J = 9.0 Hz, 2H), 7.86 (d, J = 8.6 Hz, 2H); ESI$^+$ MS m/z 341.1 $(M + H)^+$, 358.1 $(M + NH_4)^+$.

Methyl 2-(4-Aminobenzenesulfonyl)amino-6-methoxyhex-4-ynoate (22). Jone's Reagent (8 N, 1.2 mL) was added at room temperature to a solution of **21** (3.1 g, 9.1 mmol) in acetone (75 mL). Once the reaction was complete by TLC it was quenched by addition of 2-propanol (10 mL) and the green precipitate was filtered off through a pad of Celite. The acetone was evaporated off and the green residue was partitioned between EtOAc, and 5% aqueous $NaHCO_3$. The basic layer was acidified with concentrated HCl and then extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered and evaporated to yield a tan solid (2.27 g) which was dissolved in MeOH (40 mL) and to this was added concentrated H_2SO_4 at room temperature. The reaction stirred 24 h and then neutralized with Na_2CO_3. The MeOH was evaporated and the remaining aqueous layer was extracted with EtOAc, which was dried with $MgSO_4$, filtered and evaporated to a yellow oil (1.92 g, 65%) which was used without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.72−2.75 (m, 2H), 3.35 (s, 3H), 3.64 (s, 3H), 4.04−4.10 (m, 3H), 5.36 (d, J = 8.79 Hz, 1H), 6.69 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H); ESI$^+$ MS m/z 327.1 $(M + H)^+$, 344.2 $(M + NH_4)^+$.

Methyl 2-{[4-(4-Bromobenzoyl)amino]benzenesulfonyl}-amino-6-methoxyhex-4-ynoate (23). *p*-Bromobenzoyl chloride (296 mg, 1.3 mmol) was added at room temperature to a solution of **22** (400 mg, 1.2 mmol) in CH_2Cl_2 (10 mL) and NEt_3 (4 mL) and stirred 12 h. The soluion was diluted with EtOAc and washed with 0.5 N HCl. The EtOAc layer was dried with $MgSO_4$, filtered and evaporated to a yellow solid which was recrystallized from CH_2Cl_2 and hexanes to yield a white solid (270 mg, 44%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.76−2.81 (m, 2H), 3.36 (s, 3H), 3.66 (s, 3H), 4.05 (s, 2H), 4.12−4.18 (m, 1H), 5.46 (d, J = 9.0 Hz, 1H), 7.66−7.72 (m, 2H), 7.75−7.85 (m, 4H), 7.87−7.92 (m, 2H); ESI$^+$ MS m/z 509.0 $(M + H)^+$.

2-{[4′-Methoxy(1,1′-biphenyl)-4-yl]sulfonyl}amino-5-(3-morpholinophenyl)pent-4-ynoic Acid (28). The methyl ester **10** (250 mg, 0.468 mmol) was taken in 28 mL methanol and treated with KOH (473 mg, 8.43 mmol) in 3 mL of water, then stirred overnight at 25 °C. The methanol was evaporated from the crude and NaH_2PO_4 solution (pH = 6) added and extracted with EtOAc. The EtOAc layer was washed two times with NaH_2PO_4 and once with brine, then dried over Na_2SO_4, filtered and evaporated. The material was absorbed onto silica gel and purified over a silica column eluting with hexane: EtOAc (1:1) followed with EtOAc and finally with EtOAc: MeOH (8:2). Product fractions were combined and evaporated to give 186 mg (77%) of tan powder: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.72−2.88 (m, 2H), 3.06−3.18 (m, 4H), 3.70−3.71 (m, 4H), 3.80 (s, 3H), 4.00−4.08 (dd, J = 7.1, 14.3 Hz, 1H), 6.76−6.78 (d, J = 7.1 Hz, 1H), 6.77 (s, 1H), 6.86−6.90 (d, J = 8.6, 1H), 7.04−7.07 (d, J = 8.8 Hz, 2H), 7.14−7.20 (t, J = 7.5, 1 H), 7.64−7.66 (d, J = 8.8 Hz, 2H), 7.74−7.77 (d, J = 8.3 Hz, 2H), 7.85−7.88 (d, J = 7.1 Hz, 2H); ESI$^+$ MS m/z 521.0 $(M + H)^+$. Anal. ($C_{28}H_{28}N_2O_6S \cdot 0.5H_2O$) C, H, N.

MMP Inhibition Assay. The preparation of the human recombinant MMPs used in these studies has been described previously.[19] MMP inhibitors were tested for their ability to inhibit human MMPs using the quenched fluorescence assay.[37] This assay was modified to fit a 96-well format to increase the throughput. Assays for MMP-1, -3, -8 and -13 employed human recombinant truncated enzymes. MMP-7 was assayed using recombinant catalytic domain. The optimal amount of each enzyme to produce significant and reproducible substrate cleavage was determined in preliminary experiments. Assays for MMP-2 and -9 utilized human recombinant full-length enzymes. MMP-2 was activated by incubating proMMP-2 with APMA (1 mM) for 45 min. The final concentration of MMP-2 in the assay mixture was 1 nM. ProMMP-9 was activated with MMP-3 (ratio 20:1) for 2 h and diluted to a final assay concentration of 0.75 nM. The final concentration of MMP-3 in the assay was 0.038 nM. The low concentration of MMP-3 in the final MMP-9 dilution did not contribute to the rate of substrate cleavage as assessed by control experiments with 0.038 nM MMP-3. MMP-1, -3, -7, -8, and -13 were used at final concentrations of 8, 16, 2, 4 and 0.5 nM, respectively. The MMP assays was performed using the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 at a concentration of 4 μM at 25 °C. The assay buffer was 50 mM Tris, pH 7.5, 0.2 M NaCl, 5 mM $CaCl_2$ and 0.02% Brij-35. The increase in fluorescence due to cleavage of the substrate (Gly−Leu bond) was monitored kinetically for 30 min with a BMG Fluostar fluorescence plate reader (λ_{ex} 328 nm, λ_{em} 393 nm). Each 96-well microtiter plate contained 100 μL of substrate and 50 μL of enzyme in each well. 50 μL of MMP inhibitor was added to each well (except for positive control) to give a final volume of 200 μL/well. MMP inhibitors were tested at 8 different concentrations and an IC_{50} was calculated using the formula: $V_i/V_o = 1/1 + [I]/IC_{50}$, where V_i is the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I] and V_o is the initial velocity in the absence of inhibitor.

BU Solubility Determination. Approximately 250 μL of pH 7 0.05 M sodium phosphate buffer (ionic strength 0.15 M with NaCl) was placed in a 1-mL HPLC vial. A small amount (3∼4 mg) of compound was then added to the buffer. The solution was sonicated for about 1 min to dissolve the test compound. The solution was then set on a wrist-action shaker and shaken for 24 h at room temperature. If necessary, more compound was added to achieve a saturated solution after agitation. The saturated solution was transferred into a 1.7-mL conical micro centrifuge tube and centrifuged for 8 min. An aliquot of the saturated solution was withdrawn and diluted using mobile phase (methanol:0.1% formic acid). The sample was assayed against known standards. These standards were prepared using the test compound from concentrations of 1 to 250 μg/mL dissolved in identical mobile phase.

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Supporting Information Available: Additional physical data (^{1}H NMR, mass, and analytical) on final compounds not previously disclosed. This material is available free of charge via the Internet at http://pubs.acs.org.

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